REMARKS

Claims 1-3, 5, 7-11 and 14 are pending in the application.

Reconsideration of the application is respectfully requested.

Rejections under 35 U.S.C. Section 103

(a) Rejection of claims 1-2, 7, 10-11 and 14

Claims 1-2, 7, 10-11 and 14 stand rejected as obvious over Paulson *et al.* (US 5,858,751) in view of Hellman *et al.* (1995) and Clark *et al.* (2001) on the following grounds:

(i) Paulson *et al.* teach methods and expression of eukaryotic α(2,3) sialyltransferase (ST3Gal3) proteins in prokaryotic organisms;

(ii) Clark *et al.* teach methods of isolation, purification and refolding of insoluble protein from inclusion bodies using redox buffers; and

(iii) Hellman *et al.* teach solubilizing insoluble protein from inclusion bodies using protein fused to maltose binding domain (MBD).

The Examiner states that it would have been obvious to combine the teachings of the cited references and that "the expectation of success is high, because the above cited references define the status of the prior art in the successful method of expression, isolation, solubilization and refolding of sialyltransferase from inclusion body of prokaryotes expression systems" [Official Action mailed March 6, 2009, page 5, 1st par.]. Applicants respectfully traverse the rejection.

Applicants respectfully submit that even if a person of skill in the art would have had a reason to modify the teachings of the cited art to recombinantly produce eukaryotic α(2,3) sialyltransferase (ST3Gal3) of Paulson using methods of Clark and Hellman, s/he would not have had reasonable expectation of success that a solubilized *and* refolded ST3Gal3 protein comprising a maltose binding protein starting from an insoluble protein, as recited in the present claims, could have been produced. Based on general approaches taught by the prior art, as discussed in more

detail below in the context of recent US Supreme Court's decision, *KSR International Co. v. Telefex Inc.*, 550 U.S. _, 82 U.S.P.Q.2d 1385 (2007), and subsequent Federal Circuit Court decisions dealing with inventions in life sciences arts, producing solubilized and refolded α(2,3) sialyltransferase (ST3Gal3) protein according to methods recited in the present claims would not have been predictable.

In *KSR,* the Supreme Court "reaffirmed the familiar framework for determining obviousness as set forth in *Graham v. John Deere Co.*, but stated that the Federal Circuit had erred by applying the teaching-suggestion-motivation (TSM) test in an overly rigid and formalistic way." *Examination Guidelines for Determining Obviousness Under 35 U.S.C. 103 in View of the Supreme Court Decision in KSR International Co. v. Teleflex Inc.*, 72 Fed. Cir. 57526, par. bridging cols. 2-3, [hereinafter "Guidelines"] citing *KSR*, 82 USPQ2d at 1391.[1] The issue in *KSR* was obviousness of a mechanical invention which represented a combination of elements found in the prior art. Importantly, the Supreme Court recognized that predictability was a necessary component of obviousness analysis and stated that a combination of known elements would have been obvious if "the combination would have yielded nothing more than predictable results to one of ordinary skill in the art at the time of the invention." *KSR*, 82 USPQ2d at 1395 (emphasis added); *see also* Guidelines at 57529, 2nd col.

Subsequently, Federal Circuit Court applied the holding of *KSR* to less predictable, life sciences inventions. *E.g. Eisai Co. v. Dr. Reddy's Labs.,* 533 F.3d 1353 (Fed. Cir. 2008). In *Eisai,* the Court recognized that *KSR* contemplated instances where "some reasons for narrowing the prior art universe to a 'finite number of identified, predictable solutions'" could exist but stated that chemical arts are often unpredictable and "KSR's focus on these 'identified, predictable solutions' may present a difficult hurdle because potential solutions are less likely to be genuinely predictable." *Eisai*, 533 F.3d at 1359. With this as a backdrop, the Federal Circuit Court held that the claimed compound was not obvious over the prior art compound even though the claimed compound differed from the one in prior art only by one subsistent. *See also, Sanofi-Synthelabo v. Apotex, Inc.*, 550 F.3d 1075 (Fed. Cir. 2008) (holding that disclosure of the racemic mixture did not

[1] Applicant does not provide copies of cases cited herein or of the Guidelines as they are likely to be readily available to the Examiner. Should the Examiner require a copy, please telephone the undersigned.

make the isolated racemate *prima facie* obvious); *P&G v. Teva Pharms. USA,* 2009 U.S. App. LEXIS 10475 (Fed. Cir. 2009) (holding that a positional isomer was not obvious over the prior art compound).

Accordingly, based on *KSR*, as explained by subsequent Federal Circuit Court decisions, to be obvious, the present claims must have been predictable from the combined teachings of cited references. Due to the nature of the art, and evidence presented herein, this is not the case.

With respect to Paulson, Applicants submit that Paulson primarily focuses on cloning and sequence analysis of mammalian (*e.g.*, porcine, rat, human) sialyltransferases. When Paulson discloses production of recombinant sialyltransferases, it is in eukaryotic expression systems (*e.g.* monkey kidney cells (COS), insect cells (Baculovirus system)) (*see*, Paulson, *e.g.*, Example 3, 6, 7, 13). Paulson mentions prokaryotes with reference to cloning vectors and discloses recombinant protein production in prokaryotic expression systems only in a general (text-book) manner (*see*, Paulson, column 11, lines 25-66). He also states generically that "alternatively, unglycosylated sialytransferase is produced in recombinant prokaryotic cell culture." (*see*, Paulson, column 10, lines 38-40, emphasis added). Based on Paulson's disclosure, a person of skill in the art would not have known whether the unglycosylated protein he mentions was produced, and if so, whether it was produced in soluble or insoluble form and, if insoluble, whether it could have been refolded as recited in Applicants' present claims.

Clark and Hellman do not remedy Paulson's deficiencies, and in combination with Paulson, do not provide reasonable expectation of success of arriving at the present invention, for the reasons discussed below:

With respect to Hellman, Applicants note that: (i) Hellman discloses experiments with a deletion mutant of cyclomaltodextrin glucanotansferase (ΔssCGT), which protein is different from α(2,3) sialyltransferase (ST3Gal3) recited in Applicants' present claims; and (ii) Hellman admits that "each protein seems to require a specific denaturation-refolding pathway to give a maximal yield of functional molecules" (*see*, Hellman, page 56, column 2, top paragraph, emphasis added). Further, Hellman concluded MBD fusion to ΔssCGT "did *not* significantly affect the *in vitro* folding." (*see*, Hellman, page 56, abstract, last sentence, emphasis added). Therefore, based on

Hellman's disclosure a person of skill in the art would not have been able to appreciate what the effect, if any, of MBD fusion would have been on protein refolding. In other words, from Hellman, a skilled individual would not have known whether preparing an MBD fusion of a specific type of protein, *e.g.*, $\alpha(2,3)$ sialyltransferase (ST3Gal3) recited in Applicants' present claims, would have had any effect (adverse or beneficial) on solubilization and refolding. In fact, Applicants have unexpectedly discovered that "MBP domains can enhance refolding of insoluble eukaryotic glycosyltransferases after solubilization of the proteins from *e.g.*, an inclusion body" (*see*, specification, page 36, lines 13-15). Additionally, Applicants have shown that "refolded MBP-ST3GalIII enzymes were *more active* in transfer of sialic acid to a glycoprotein acceptor molecule" than refolded GST-ST3GalIII (*see*, specification, page 76, lines 21-22, emphasis added).

Clark is a review article which discloses general approaches for protein refolding for industrial processes, but Clark does not disclose results for any particular protein. Clark mentions reduced and oxidized glutathione (GSH/GSSG) and cysteine/cystine as oxido-shuffling reagents. However, it was also known in the art that GSH/GSSG can negatively affect the production of correctly folded target proteins. For example, Winter *et al.* showed that "the addition of glutathione had a negative effect on the yield of native proinsulin" thus suggesting unpredictability of success with glutathione in redox systems (*see*, Attachment 1- Winter *et al.*, *Increased production of human proinsulin in the periplasmic space of Escherichia coli by fusion to DsbA*, Journal of Biotechnology, 2000, 84:175-185; page 183, column 2, lines 17-24).

In short, the combination of cited references, at best, merely suggests pursuing a "general approach that seemed to be a promising field of experimentation" (*see Pfizer v Apotex*, 480 F.3d 1348 (Fed. Cir. 2007) citing *In re O'Farrell*, 853 F. 2d 894, 903 (Fed. Cir. 1988)). This is not a standard for obviousness.

To emphasize the importance of testing general strategies for re-folding for a particular type of protein to achieve functional recombinant protein, Applicants attach a publication which discusses contemporaneous knowledge of a person of skill in the art at the time of filing of the present application (*see*, Attachment 2- Fahnert B., *Folding-promoting agents in recombinant protein production*, Methods in Molecular Biology, 2004, 267:53-74). While certain general approaches for promoting correct folding of recombinant proteins were known, the main theme of

the article was that "the impact of all these strategies cannot be predicted and must be analyzed and optimized for the corresponding target protein" (Attachment 2, Fahnert, page 53, Summary, emphasis added). Fahnert had reviewed many protocols published over the years and concluded that "it becomes clear quite soon that as every target protein is different, one cannot predict the effect of a certain approach. Most of the time one cannot even speculate" (see, Attachment 2, Fahnert, page 60, last paragraph).

Therefore, in view of the nature of the art, a person of skill in the art would not have been able to reasonably predict the outcome of following general approaches of Paulson, Hellman and Clark for MBD-ST3Gal3 protein. Moreover, as shown in the specification and discussed above, the methods recited in pending claims have the unexpected benefit of producing a more active enzyme in comparison to other approaches.

Withdrawal of the rejection is respectfully requested.

(b) Rejection of claims 3 and 5

Claims 3 and 5 stands rejected as obvious over the combination of references cited above and further in view of Ramakrishnan *et al.* (2001) on the above-mentioned grounds and further on the ground that Ramakrishnan teaches that "mutation of unpaired cysteine, Cys 342 to Thr of a beta-galactosyltransferase resulted in 2 to 3 fold increase in yield of refolded enzyme" [Office Action, page 6, 3rd full paragraph]. Applicants respectfully traverse the rejection.

Claims 3 and 5 are patentable over the prior art for the reasons, and in view of evidence, presented above and further because Ramakrishnan, who worked with a protein different from MBD-α(2,3) sialyltransferase (ST3Gal3), fails to provide any additional or specific guidance to arrive at the present invention. Without more, a person of skill in the art would not have been able to reasonably predict the outcome of following approaches of the cited art.

Withdrawal of the rejection is respectfully requested.

(c) Rejection of claim 5

Claim 5 stands rejected as obvious over Paulson *et al.* in view of Hellman *et al.* and Clark *et al.* and further in view of Nilsson *et al.* on the grounds mentioned above and further on the ground that Nilsson *et al.* teach the use of affinity tags (*e.g.,* glutathione-S-transferase (GST), poly-His domains) for purification of proteins using affinity columns. Applicants respectfully traverse the rejection.

In view of the fact that Nilsson is a general review of affinity fusion strategies and does not disclose or suggest MBD-α(2,3) sialyltransferase (ST3Gal3) protein or protein refolding strategies, Applicants submit that Nilsson's disclosure does nothing to remedy the deficiencies of the references discussed above. Thus, for the reasons, and in view of evidence, presented above Nilsson in combination with Paulson, Hellman and Clark fails to provide reasonable expectation of success at arriving at the invention of claim 5.

Withdrawal of the rejection is respectfully requested.

(c) Rejection of claims 8 and 9

Claims 8-9 stand rejected as obvious over Paulson *et al.* in view of Hellman *et al.* and Clark *et al.* on the grounds mentioned above, and on the ground that Paulson teaches that "such sialytransferases may be employed in multienzyme systems" (*see,* Paulson, column 4, line 28-30). Applicants respectfully traverse the rejection.

Applicants submit that Paulson's disclosure of multienzyme systems is in the context of their function for "synthesis of oligosaccharides and their derivatives." (*see,* Paulson, column 4, line 30), and not in the context of refolding multiple soluble enzymes in one reaction mixture. While use of mutlienzyme systems may suggest desirability of producing active enzymes together in one reaction mixture, a person of skill in the art would have had, for the reasons and evidence presented above, even less expectation of success than with a single enzyme. Withdrawal of the rejection is respectfully requested.

Conclusion

In view of the above remarks and evidence, Applicants believe that the application is in condition for allowance. Such action is respectfully requested.

Respectfully submitted,

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